determined by localized variations in their amino-acid sequence, rather than by architectural constraints, function type or the organism's environment.
Here, we use experimental and computational methods to quantify and rationalize the ion specificity of a rotor ring from an F-type ATP synthase. Specifically we employ Isothermal Titration Calorimetry to experimentally determine the H^+/Na^+-selectivity of the rotor ring from *Ilyobacter tartaricus*, and find this ring to be selective for H^+ over Na^+ by three orders of magnitude. This result explains the observation that this ATP synthase is functionally Na^+-specific, owing to the much larger concentration of Na^+ over H^+ under physiological conditions. Using free-energy molecular simulations, we then compare the *I. tartaricus* ring with that of the *Enterococcus hirae* V-type ATPase, which is known to be essentially non-selective (and thus also coupled to Na^+), as well as with that of *Spirulina platensis*, whose H^+ selectivity is at least a billion-fold. Taken together with previous theoretical studies, this analysis establishes the general principle underlying the broad spectrum of H^+/Na^+ selectivities in the rotary ATPase family, which might also be applicable to other membrane transport systems.

986-Plat
Recent Structures and Molecular Dynamics Simulations Offer New Perspective on Na^+/H^+ Antiporters
David L. Dotson[1], Chiara Lee[2], Shoko Yashiro[2], Povilas Uzdavinys[3], Christoph von Ballmoos[3], David Drew[3], Alexander D. Cameron[4], Oliver Beckstein[1].
[1]Department of Physics, Arizona State University, Tempe, AZ, USA, [2]Division of Molecular Biosciences, Imperial College London, London, United Kingdom, [3]Centre for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, [4]School of Life Sciences, University of Warwick, Coventry, United Kingdom.
Na^+/H^+ antiporters are vital to cells for maintaining homeostasis, especially in high-salt environments. New crystal structures for two such antiporters, cytoplasmic-open Escherichia coli NhaA and periplasmic-open Thermus Thermophilus NapA, show high structural similarity despite low sequence identity. Among their common features are a set of highly conserved charged residues (three aspartates and one lysine) near the putative ion binding site. Using molecular dynamics simulations, we observe that interaction with sodium is dependent on the charge states of the conserved aspartates. The lysine and the nearest asparate also form a previously unidentified salt bridge. Under simulated physiological pH the presence of a sodium ion disrupts and breaks the salt bridge in NhaA. Given the presence of this salt bridge in both protein structures, its behavior under simulation, and the known importance of the conserved lysine, we hypothesize that this salt bridge is directly involved in ion binding and transport. To address the question of sodium binding, we performed an ensemble of all-atom equilibrium molecular dyanamics simulations (over 10 μs in total), varying the protonation states of the conserved residues and quantifying the resulting sodium interaction. To address the question of proton binding, we performed heuristic pKa calculations on this ensemble of simulations, which support the hypothesis that the lysine binds protons in a sodium-dependent manner. In order to elucidate the structural basis for alternating access in NhaA, we created an outward-facing model of NhaA, based on the periplasmic-open NapA structure, and simulated conformational transitions of NhaA from its cytoplasmic- to periplasmic-open state using dynamic importance sampling MD. Taken together, the combination of recent structural and dynamic simulation data suggests a new model of ion binding and transport for the CPA2 class of antiporters.

987-Plat
Understanding Selectivity of the Na^+/K^+ -ATPase using a Computational Approach
Asghar M. Razavi[1], Vincenzo Carnevale[2], Lucie Delemotte[2], Vincent A. Voelz[1].
[1]Chemistry, Temple University, Philadelphia, PA, USA, [2]BioChemistry, Temple University, Philadelphia, PA, USA.
The Na^+/K^+-ATPase (NKA) is a membrane protein that transports Na^+ ions out of the cell and K^+ ions into it against their concentration gradient. The high selectivity of NKA for Na^+ or K^+ can be explained by distinct affinities of the two ionic species for the binding sites, by different kinetic barriers encountered to reach them, or by both. Understanding the exact location of binding sites is the first step in determining the origin of selectivity in NKA. We used molecular dynamics simulations and free energy calculations to study ion binding. We show that the exact location of the binding sites, the thermodynamic affinity of the two ions and the cooperativity of the binding depend crucially on the protonation state of the binding residues. Importantly, this protonation state changes when the NKA shifts between the sodium and potassium occluded states.

988-Plat
X-Ray Crystallographic Study of Na,K-ATPase in Complex with Cardiotonic Steroids
Haruo Ogawa[1], Kanna Motoyama[1], Flemming Cornelius[2], Bente Vilsen[2], Chikashi Toyoshima[1].
[1]Center for Structural Biology of Challenging Proteins, IMCB, The University of Tokyo, Tokyo, Japan, [2]Department of Biomedicine, Arhus University, Arhus, Denmark.
Na,K-ATPase transports three sodium ions from the cytoplasm to the extracellular side and counter-transports two potassium ions upon hydrolysis of one ATP in each reaction cycle, thereby establishing gradients for sodium and potassium ions across the membrane. These ion gradients are used in many fundamental processes, notably, excitation of nerve cells. It is one of the most important members of the P-type ATPases. It is a hetero-trimer complex, consisting of alpha-subunit (112K), beta-subunit (55K) and FXYD regulatory protein (10K). Na,K-ATPase is also regarded as a membrane receptor for cardiotonic steroids (CTS), including digoxin prescribed for congestive heart failure and supraventricular arrhythmias for ~200 years. Moreover, CTS have been studied as potential drugs for cancer chemotherapy, because they inhibit growth of cancer cells through the binding to Na,K-ATPase. Thus, the elucidation of the binding mechanism of CTS to Na,K-ATPase has direct medical implication. We reported a crystal structure with bound ouabain at 2.8 Å resolution (Ogawa et al., PNAS 2009), but the affinity of ouabain under the conditions employed is low due to antagonism with bound potassium. As to the high affinity ouabain-bound form, a crystal structure was reported very recently by other group, but at only 3.4 Å resolution with a very low completeness (Laursen et al., PNAS 2013). Thus, higher-resolution structures are still awaited. Moreover, structures bound with other CTSs having different sugar moieties and/or different lactone rings are also awaited. Here, we report our recent crystal structures with bound several CTSs, including different sugar moieties and lactone rings, at better than 3.0 Å resolution. These structures provide detailed information on the interaction of Na,K-ATPase and CTS and may lead us to design better drugs for treating heart failure, arrhythmias and cancer.

989-Plat
Identification of Structural Motifs in P-Glycoprotein Responsible for the Drug-Mediated Inhibition of ATP Hydrolysis
Eduardo E. Chufan, Khyati Kapoor, Suresh V. Ambudkar.
National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.
P-glycoprotein (P-gp, ABCB1) is the most studied member of the ATP-Binding Cassette (ABC) transporter superfamily. This transporter utilizes energy from ATP hydrolysis for the efflux of a great variety of compounds, including anticancer drugs. P-gp is expressed at the apical surface of epithelial cells in the intestines, kidney, liver, adrenal gland, blood-brain barrier, and placenta, and therefore affects the pharmacokinetics of many drugs. The expression of P-gp at the surface of tumor cells is also one of the mechanisms of multidrug resistance, which occurs in many cancers. The development of an effective inhibitor requires first the understanding of the mechanism of action of P-gp. In our recent work, mutagenesis and molecular modeling studies led to the identification of a pair of phenylalanine-tyrosine structural motifs that mediate the inhibition of ATP hydrolysis by drugs such as zosuquidar, tariquidar and elacridar. Upon mutation of any of these residues, drugs that inhibit the ATPase activity of P-gp switch to stimulation of the activity. Molecular modeling revealed that the phenylalanine residues interact with the tyrosines in an edge-to-face conformation, helping the tyrosines to adopt the proper orientation to effectively establish hydrogen-bond contact with the inhibitor. Biochemical investigations along with transport studies in intact cells showed that the inhibitors bind at a high affinity site to produce inhibition of ATP hydrolysis and transport function. Upon mutation of tyrosine or phenylalanine residues that disrupts the structural motifs, they bind at lower affinity sites, leading to stimulation of ATP hydrolysis and poor inhibition of transport.

990-Plat
The Hydrolysis Cycle of ATP-Binding Cassette Nucleotide-Binding Domains
Srinivasan Krishnan, Maria E. Zoghbi, Guillermo A. Altenberg.
Cell Physiology and Molecular Biophysics, Texas Tech University Health Sciences Center, Lubbock, TX, USA.
ATP-binding cassette (ABC) proteins have two nucleotide binding domains (NBDs) that work as dimers to bind and hydrolyze ATP. ATP binding elicits association of the two NBDs, with the NBDs in a head-to-tail arrangement and two nucleotides "sandwiched" at the dimer interface. Each of the two